Environmental Health Perspectives
Vol. 72, pp. 39-44, 1987

Biological Effects of Short-Term, High-Concentration Exposure to Methyl Isocyanate. V. Morphologic Evaluation of Rat and Guinea Pig Lungs

by Edward H. Fowler,* Darol E. Dodd,* and Catherine M. Troup*

The morphologic changes induced in the lungs of rats and guinea pigs exposed to high concentrations of MIC vapor (100, 600, and 1000 ppm in the rat and 25, 125, 225, and 675 ppm in the guinea pig) for a short time (15 min) in a static exposure chamber were evaluated at varying postexposure periods (0, 1, 2, 4, and 16 hr). The 675 ppm-exposed guinea pigs were evaluated only immediately following removal from the chamber. Attention was primarily focused on the intrapulmonary conducting airways and the parenchyma (gas exchange region) of the lungs. The severity of morphologic changes observed by light microscopy was directly correlated with exposure concentration and time postexposure in both species. Specifically, degenerative changes were observed in the bronchial, bronchiolar, and alveolar epithelium in both species. Quantitative differences were observed; 100 ppm of MIC in the rat resulted in much less damage than did 125 ppm of MIC in the guinea pig. Morphologic evidence of sloughing of large sheets of conducting airway epithelium with fibrin buildup and increased mucus production resulted in plugging of major airways and atelectasis. These observations support the hypothesis that tissue hypoxia was a major contributing factor resulting in death.

Introduction

Methyl isocyanate (MIC) is a highly reactive chemical which causes severe necrosis of the mucosae lining the respiratory tract. The nasal passages, larynx, trachea, and larger conducting airways in the lungs underwent necrosis when rats, mice, or guinea pigs were exposed to concentrations as high as 20.4 ppm following an acute dynamic exposure of 6 hr duration (*1,2*). Guinea pigs were more sensitive to MIC than rats, and rats were more sensitive than mice.

Subsequent to the incident which occurred in Bhopal, India, on December 3, 1984, Union Carbide initiated additional studies at the Bushy Run Research Center. These studies, designed to complement the studies being conducted by NTP of NIEHS (see papers in this issue), concentrated on short exposure to high vapor concentrations of MIC in the two species which had proven most sensitive in previous studies, and in which qualitative morphologic differences had been found, namely, the rat and guinea pig (*1,2*). Much of our previous work indicated that the principal region affected following either single or repeated 6-hr exposures was the mucosa of the upper respiratory tract, as well as the conducting airways of the lung (*1–4*). Consequently, we elected to focus attention on the morphologic changes induced by MIC on the conducting airways of the lung, as well as on the pulmonary parenchyma, or gas exchange regions. An ultrastructural evaluation of the lungs will be submitted at a later time.

Materials and Methods

Specific pathogen-free (SPF) Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, IN) and SPF Hartley strain guinea pigs (Hazelton-Dutchland Laboratories, Inc., Denver, PA) were exposed to MIC vapor concentrations ranging from 25 to 3000 ppm for short intervals (averaging 15 min) in a static exposure chamber (*3*). Following removal from the chamber, the animals were either sacrificed immediately (0 hr) or allowed to survive for intervals of 1, 2, 4, or 16 hr before sacrifice. A few animals were allowed to live up to 24 hr. Many animals died following exposure, especially those exposed to concentrations of 225 ppm or higher; guinea pigs died at lower concentrations than rats (*5*).

Blood samples were obtained either from anesthetized animals prior to sacrifice or immediately following

*Bushy Run Research Center, Union Carbide Corporation, R. D. 4, Mellon Road, Export, PA 16532.

sacrifice for hematologic and blood chemistry analyses (*6*). Blood was also obtained from selected guinea pigs for determination of complement activity. Limited tissues (lungs, heart, liver, kidney, and in some cases, spleen) were collected immediately following sacrifice, or death, if they could be harvested shortly after death.

Tissues from 97 rats exposed to target MIC concentrations of 0 (11), 100 (28), 600 (25), and 1000 (33) ppm and from 75 guinea pigs exposed to target concentrations of 0 (12), 25 (18), 125 (21), and 225 (24) ppm for 15 min were evaluated for this report. For all exposure groups, tissues were evaluated by light microscopy from animals sacrificed at 0, 1, 2, 4, and 16 hr following termination of exposures.

Four pairs of guinea pigs were exposed to a target MIC concentration of 675 ppm, with the expectation that one of the guinea pigs would die during exposure (*5*). When a death occurred, both guinea pigs were removed from the exposure chamber as rapidly as possible for collection of blood for complement activity (*10*). No guinea pigs exposed at this concentration were allowed to survive following exposure. The lungs from eight of these guinea pigs were examined by light microscopy.

The tissues collected for light microscopy were placed in 10% neutral buffered formalin for at least 24 hr before being trimmed for processing. Tissues were trimmed to include a portion of each of the lung lobes, major conducting airways, several sections through the heart, as well as cross or longitudinal sections of liver, kidneys (both), and spleen. The tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H & E).

Results

Rats

No biologically important morphologic changes were found by light microscopy in tissues other than the lungs. A description of the changes associated with specific MIC exposure concentrations follows.

100 ppm. In comparison to control rats, the most striking change was the immediate perivascular infiltrate of neutrophils (Fig. 1). Lungs from rats sacrificed at 0 hr had evidence of slight bronchiolar epithelial damage consisting of increased acidophilia (eosinophilia) of the cytoplasm and decreased cell contact.

At 1 hr postexposure, perivascular neutrophils were still prominent. Bronchiolar epithelial cells were observed lifting away from the basement membrane in large sheets. At 2 hr, congestion and some foci of hemorrhage were found in the lungs, and at 4 hr postexposure, fibrin was present in some of the alveoli.

At 16 hr postexposure, in addition to the perivascular edema, alveolar fibrin, focal hemorrhage, and neutrophils in the interstitial area, large plugs of fibrin and cellular debris were present in some of the conducting airways.

600 ppm. Bronchiolar epithelium was observed lifting from the basement membrane in sheets immediately following the 15-min exposure (0 hr), and cellular debris was present in the bronchioles. Red blood cells were found in some alveoli, and neutrophils were observed in alveolar capillaries. By 1 hr postexposure, the airway epithelium was necrotic and perivascular edema was observed. Alveolar edema occurred in the lungs of rats sacrificed 2 hr following exposure. Pseudomembranes consisting of fibrin and cellular debris were seen in bronchi and large bronchioles of rats sacrified 4 hr following exposure. These pseudomembranes were more prominent and larger in those rats sacrificed at 16 hr postexposure. Pleural effusion and fibrin accumulation on the pleura, along with large areas of alveolar fibrin and edema, were observed in rats sacrificed 16 hr postexposure. Degeneration of alveolar epithelial and endothelial cells was apparent with light microscopy.

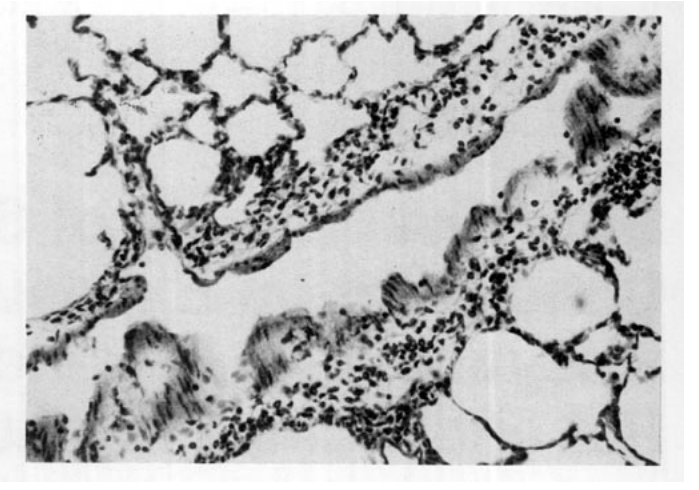

FIGURE 1. Perivascular neutrophilic infiltrate in the lung of a rat sacrificed immediately following 15 min of exposure to 100 ppm of MIC vapor. H & E, × 160.

1000 ppm. The lungs of rats exposed to 1000 ppm for 15 min had evidence of alterations that were more severe and appeared earlier than those seen in the lower exposure concentration groups. The bronchiolar epithelium was observed lifting away from the basement membrane immediately following termination of exposures (Fig. 2). In addition, perivascular and interstitial neutrophils were prominent, RBCs were found in a few alveoli, and congestion together with peribronchial and perivascular edema were observed. At 1 hr postexposure, bronchioles were plugged with fibrin and cellular debris that included neutrophils (Fig. 3).

Fibrin was present in the alveolar spaces 2 hr postexposure (Fig. 4). Multifocal areas of hemorrhage were observed, and marked necrosis and pseudomembrane formation occurred in the airways.

At 4 hr postexposure, the lungs had evidence of marked necrosis of bronchiolar epithelium (Fig. 5). Pseudomembranes consisting of fibrin and sloughed tracheal, bronchial, and bronchiolar epithelial cells with infiltrating neutrophils were observed in the conducting airways.

Rats surviving to 16 hr postexposure had marked

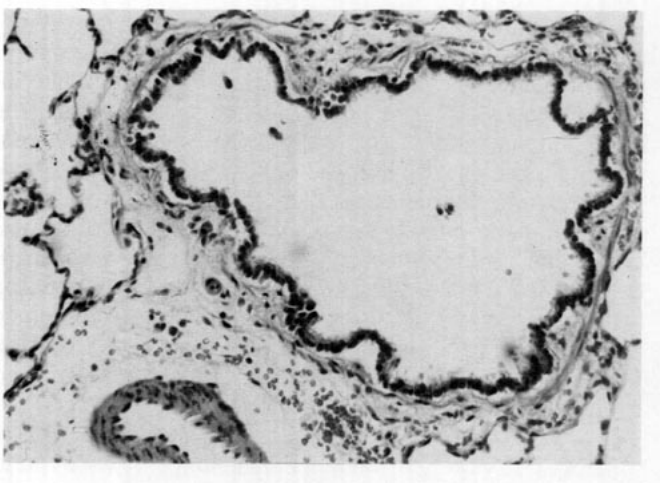

FIGURE 2. Bronchiolar epithelium lifting from its basement membrane in the lung of a rat exposed 15 min to 1000 ppm of MIC vapor and sacrificed immediately following termination of exposures. H & E, × 160.

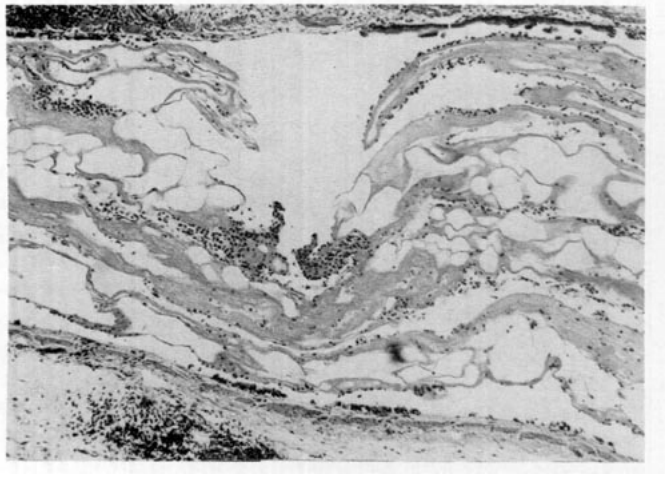

FIGURE 3. Plugging of bronchioles with fibrin and cells in the lung of a rat sacrificed 1 hr following a 15-min exposure to 1000 ppm of MIC vapor. H & E, × 63.

necrosis of airway epithelium with plugging of bronchioles. Changes in the deep parenchyma consisting of alveolar edema, fibrin deposition, and multifocal hemorrhages were observed.

Guinea Pigs

A description of the microscopic changes seen in association with specific MIC exposure concentrations follows.

25 ppm. In contrast to control animals, the lungs of guinea pigs sacrificed at 0 hr had evidence of mild bronchiolar epithelial cell degeneration characterized by small numbers of exfoliated cells in the bronchioles. In addition, mild perivascular and alveolar hemorrhage were seen. Aggregates of neutrophils were adhering within the blood vessels. At 1 hr postexposure, some bronchioles appeared collapsed and areas of atelectasis were observed. Multifocal hemorrhages and increased sequestration of neutrophils in alveolar capillaries were observed. Peribronchial edema was observed, and cellular debris was found in the bronchiolar lumina.

At 2 hr postexposure, cell debris was evident within bronchioles and alveolar spaces. Increased numbers of neutrophils were observed in capillaries, and multifocal atelectasis occurred. At 4 hr postexposure, the lesions were similar but more marked. Some alveoli appeared dilated in comparison to controls. At 16 hr postexposure, vacuolated alveolar epithelial cells could be seen with the light microscope. Hemorrhage was scattered throughout the lungs, and multifocal atelectasis was present.

125 ppm. Immediately following termination of exposures, bronchiolar epithelial degeneration with the accumulation of much cellular debris in the bronchi were observed (Fig. 6). Alveolar macrophages appeared vac-

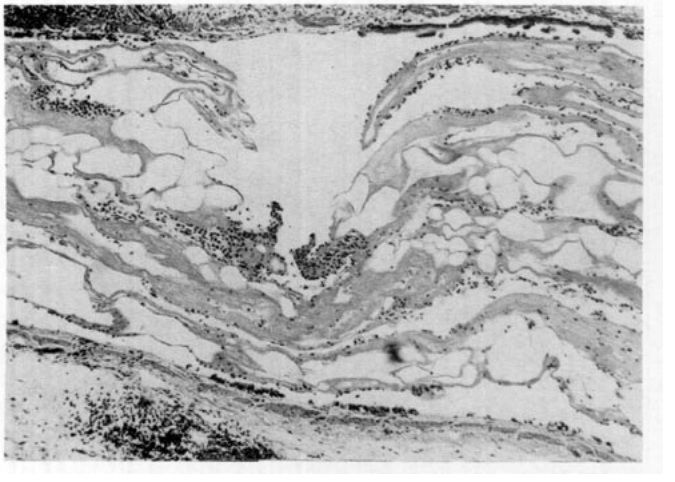

FIGURE 4. Alveolar fibrin present in a rat lung 2 hr following exposure to 1000 ppm. H & E, × 160.

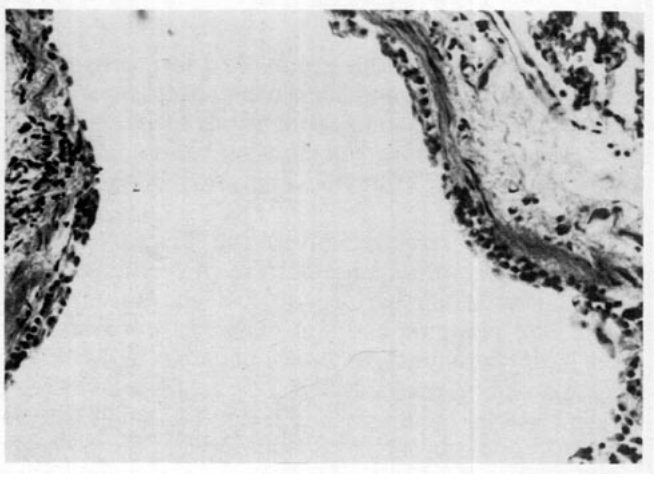

FIGURE 5. Bronchiolar epithelial necrosis in the rat lung 4 hr following a 15-min exposure to 1000 ppm of MIC vapor. H & E, × 160.

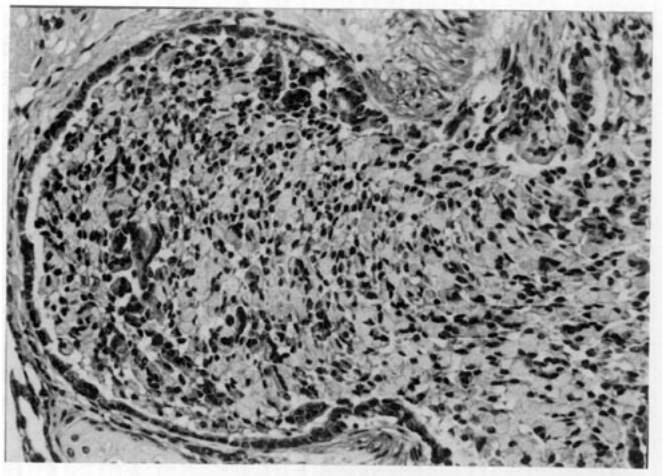

FIGURE 6. Abundant cellular debris plugging the bronchus of a guinea pig exposed 15 min to 125 ppm of MIC vapor and sacrificed immediately following termination of exposure. H & E, × 160.

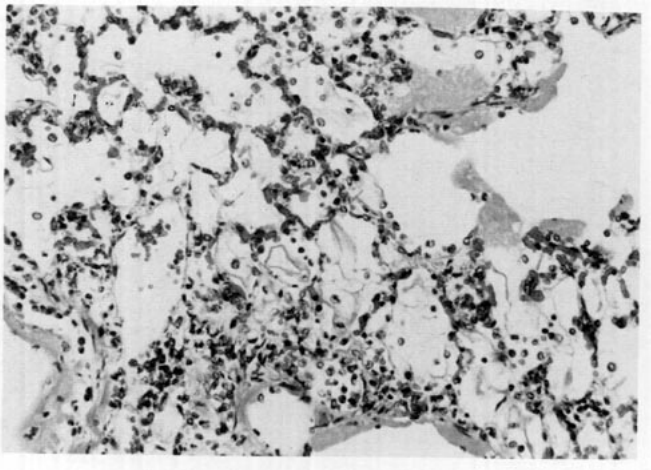

FIGURE 7. Hyaline membrane formation and alveolar fibrin and hemmorhage in the lung of a guinea pig sacrificed 16 hr following exposure to 125 ppm of MIC vapor for 15 min. H & E, × 160.

uolated in lungs from this group. At 1 hr postexposure, hemorrhage, edema, and fibrin were observed in alveoli. Neutrophils were sequestered in alveolar capillaries. Bronchiolar epithelial degeneration, pseudomembrane formation, and bronchiolitis obliterans were also observed.

At 2 hr postexposure, guinea pig lungs had considerable evidence of damage both in the conducting airways and in the lung parenchyma. At 4 hr postexposure, bronchiolar plugging and atelectasis were marked.

At 16 hr postexposure, guinea pig lungs had evidence of hyaline membrane formation (Fig. 7), in addition to alveolar edema and fibrin, alveolar epithelial cell degeneration, and bronchiolitis obliterans.

225 ppm. At the termination of exposures, fibrin and prominent cell debris, together with neutrophils, were found in the conducting airways. Bronchiolitis obliterans was also observed. Alveolar edema and increased numbers of alveolar macrophages were observed in the lung parenchyma. By 1 hr postexposure, large numbers of erythrocytes were present in the alveolar spaces. By 4 hr postexposure, a marked degree of pseudomembrane formation was seen in the conducting airways with marked multifocal alveolar edema, hemorrhage, and neutrophilic infiltration.

None of the guinea pigs exposed to 225 ppm of MIC vapor for 15 min survived 16 hr following exposure. Several were necropsied when found dead the morning after exposure, and there was evidence of damage to and plugging of the small bronchi in one of these animals (Fig. 8). Marked hemorrhage, inflammation, and congestion were also apparent.

675 ppm. Immediately following exposure to 675 ppm of MIC vapor, bronchial epithelial cells were found sloughing from the basement membrane and free in the lumina of the bronchi. Further down the conducting airways, but still in the bronchial region, the airways were occluded by both intraluminal debris and contraction of smooth muscle. Perivascular edema was present in the deep lung parenchyma, and multifocal hemorrhagic areas were found throughout the lungs.

Discussion

Nemery et al. (7) exposed rats to varying concentrations of MIC vapor in a static exposure chamber for periods up to 1 hr and reported destruction of epithelia in the upper airways. These epithelia were usually stripped from the basement membrane within 24 hr in rats exposed to lethal concentrations. They further described damage extending down into the lung parenchyma involving alveolar epithelia and endothelial cells. In the current study, the changes observed in the conducting airways of the lung, as well as the parenchyma (the alveolar ducts and alveoli), increased in severity with both exposure concentration and time after ex-

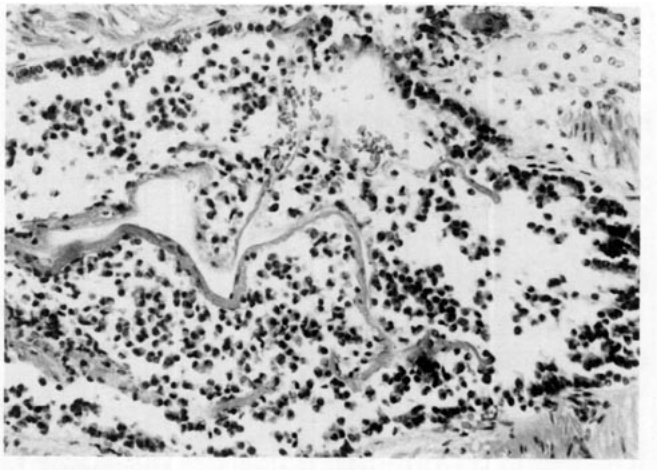

FIGURE 8. Marked inflammation and necrosis of a small bronchus has resulted in plugging the lumen of the lung in this guinea pig which died within 16 hr following a 15-min exposure to 225 ppm of MIC vapor. H & E, × 160.

posure. The changes found in the bronchioles consisted of lifting the epithelium in sheets from the basement membrane and necrosis of remaining epithelial cells. This resulted in an influx of plasma proteins and fibrin deposition leading to plugging of many of the major airways. Deep in the lung, fibrin was found in many of the alveoli, indicating damage to the alveoli.

Despite the exposure of guinea pigs to MIC vapor concentrations much lower than rats, the lesions observed in the conducting airways and lung parenchyma were more severe. Airway epithelial cell necrosis and sloughing resulted in major obstructions in some of the larger airways. As in the rat, neutrophils were sequestered in alveolar capillaries. Fragmented cellular membrane material was found in the alveolar lumina. In guinea pigs allowed to survive several hours postexposure, hyaline membranes were found along with fibrin and cellular debris in the alveoli. This supports the observation of early mortality in the guinea pig at much lower concentrations of MIC vapor when compared with the rat (*5*).

The morphologic changes induced by high exposure concentrations of MIC vapor in the lungs of rats and guinea pigs support the biological effects observed in other studies conducted by UCC (*6,8,9*). In the controlled ventilation studies (*8*), we have shown that within minutes there is an increase in intratracheal pressure with marked shifts in both blood gases and pH. These changes have been attributed to ventilation/perfusion inequality and intrapulmonary blood shunting. The changes presumably could result from failure of the air to reach the alveoli due to plugging. The present morphologic evaluation has shown that, immediately following termination of the 15-min exposures, large sheets of epithelial cells are virtually lifted from the basement membrane in the conducting airways and that these plug the more distal airways, resulting in atelectasis.

We have shown previously that MIC vapor exposure to rats, mice, and guinea pigs in a single 6-hr exposure (*1,2*), as well as repeated 6-hr exposures of rats (*3,4*), resulted in marked epithelial necrosis in the upper respiratory tract, including the nasal passages, larynx, and trachea. Even higher exposure concentrations, albeit for shorter periods, would be expected to result in exfoliation or sloughing of epithelium from these areas, leading to further blockage of the airways as a result of aspiration. In addition to the cellular debris present in the airways, large masses of fibrin and some mucus were added to the intraluminal material, resulting in further obstruction. Bronchiolitis obliterans as a result of intraluminal accumulation of debris and collapse of the bronchioles was particularly evident in guinea pig lungs, perhaps due in part to contraction of peribronchiolar smooth muscle secondary to histamine release.

The damage to epithelia throughout the respiratory tract contributes to the activation of the complement system (*10*) resulting in the sequestration of neutrophils in alveolar capillaries and infiltration into the perivascular and peribronchial connective tissue, causing an immediate peripheral neutropenia and rebound neutrophilia (*6*). However, it does not appear that the neutrophilic sequestration or infiltration is of the magnitude encountered in the animal models of adult respiratory distress syndrome in man (*11*), nor does it appear that the amount of alveolar edema or fibrin found in most of the animals examined would have produced a severe enough diffusion disturbance to prevent O_2 from reaching the blood.

Our studies were designed to terminate a few hours following exposure because of the high concentration of MIC vapor used, often well above the LC_{50} (*5*). Therefore, the intraluminal airway fibrosis described in rats and mice earlier (*1–4*) and in other studies (*12*) was not observed. We can speculate that, had animals been allowed to survive, reparative processes would have occurred, some of which would have produced fibrosis. A previous MIC repeated exposure study in rats (*3,13*) indicated that intraluminal airway fibrosis matured and contracted during the 85 days of postexposure and that interstitial fibrosis was minimal and multifocal in small areas of the lung. No morphologic studies beyond 2 weeks postexposure have been conducted in the guinea pig.

In conclusion, a short-term, high-concentration exposure of MIC vapor to rats and guinea pigs resulted in severe destruction of the epithelia lining the conducting airways resulting in sloughing of large sheets of epithelial cells which, together with fibrin and mucus, caused plugging of the more distal airways and subsequent atelectasis. This airway obstruction resulted in changes in the blood gases and pH (*8*), which resulted in a shift in the oxygen dissociation curve of hemoglobin (*9*) that very likely caused further tissue hypoxia with the resulting death of the animal.

The authors thank the inhalation staff, principally Irwin Pritts and Mac Steel, for exposing the animals used in this study, and the histology staff, particularly Gayle DiSalvo, for processing the material for light microscopy and preparing the light photomicrographs. We also thank Kathleen McCabe for typing the manuscript.

REFERENCES

1. Fowler, E. H., and Dodd, D. E. Acute inhalation studies with methyl isocyanate vapor. Part II. Respiratory tract changes in guinea pigs, rats, and mice. Fundam. Appl. Toxicol. 6: 756–771 (1986).
2. Fowler, E. H., and Dodd, D. E. Respiratory tract changes in guinea pigs, rats, and mice following a single six-hour exposure to methyl isocyanate vapor. Environ. Health Perspect. 72: 109–116 (1987).
3. Dodd, D. E., and Fowler, E. H. Methyl isocyanate subchronic vapor inhalation studies with Fischer-344 rats. Fundam. Appl. Toxicol. 7: 502–522 (1986).
4. Dodd, D. E., Fowler, E. H., Snellings, W. M., and Pritts, I. M. Methyl isocyanate eight-day vapor inhalation study with Fischer 344 rats. Environ. Health Perspect. 72: 117–123 (1987).
5. Dodd, D. E., Frank, F. R., Fowler, E. H., Troup, C. M., and Milton, R. M. Biological effects of short-term, high-concentration exposure to methyl isocyanate. I. Study objectives and inhalation exposure design. Environ. Health Perspect. 72: 13–19 (1987).
6. Troup, C. M., Dodd, D. E., Fowler, E. H., and Frank, F. R. Biological effects of short-term, high-concentration exposure to

methyl isocyanate. II. Blood chemistry and hematologic evaluations. Environ. Health Perspect. 72: 21–28 (1987).
7. Nemery, B., Dinsdale, D., Sparrow, S., and Ray, D. Effects of methyl isocyanate on the respiratory tract of rats. Br. J. Indust. Med. 42: 799–805 (1985).
8. Fedde, M. R., Dodd, D. E., Troup, C. M., and Fowler, E. H. Biological effects of short-term, high-concentration exposure to methyl isocyanate. III. Influence on gas exchange in the guinea pig lung. Environ. Health Perspect. 72: 29–33 (1987).
9. Maginnis, L. A., Szewczak, J. M., and Troup, C. M. Biological effects of short-term, high-concentration exposure to methyl isocyanate. IV. Influence on oxygen binding properties of guinea pig blood. Environ. Health Perspect. 72: 35–38 (1987).
10. Kolb, W. P., Savury, J. R., Troup, C. M., Dodd, D. E., and Tamerius, J. D. Biological effects of short-term, high-concentration exposure to methyl isocyanate. IV. *In vitro* and *in vivo* complement activation studies. Environ. Health Perspect. 72: 189–195 (1987).
11. Till, G. O., and Ward, P. A. Systemic complement activation and acute lung injury. Fed. Proc. 45: 13–18 (1986).
12. Boorman, G. A., Brown, R., Gupta, B. N., Uraih, L. C., and Bucher, J. R. Pathologic changes following acute methyl isocyanate inhalation and recovery study in B6C3F1 mice. Toxicol. Appl. Pharmacol., in press.
13. Fowler, E. H., and Dodd, D. E. Eighty-five day postexposure follow-up study in Fischer 344 rats after repeated exposures to methyl isocyanate vapor. Environ. Health Perspect. 72: 125–132 (1987).